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Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits

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Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits. The acute renal effects of hypoxemia and the ability of theophylline to prevent these effects were assessed in anesthetized and mechanically-ventilated newborn and adult rabbits. Renal blood flow (RBF) and glomerular filtration rate (GFR) were determined by the clearance of para-aminohippuric acid and inulin, respectively. Each animal acted as his own control. In 14 newborn rabbits (group 1), hypoxemia was significantly associated with a fall in GFR (−22 ± 6%) and filtration fraction (−17 ± 3%) and with an increase in renal vascular resistance (+13 ± 6%). Hypoxemia also induced a significant decline in GFR (−27 ± 6%) and RBF (−29 ± 6%) in 7 adult rabbits (group 3). Intravenous theophylline (0.5 mg/kg) completely prevented the hypoxemia-induced changes in GFR, filtration fraction (FF) and renal vascular resistance (RVR) in 8 newborn rabbits (group 2). An intravenous dose of 2.4 mg theophylline given after the induction of hypoxemia partially reversed the drop in GFR in adult rabbits (group 3). Separate renal functions were studied in 8 additional adult rabbits (group 4). Low-dose theophylline (27 μg/min) infused intra-arterially in the left kidney partially prevented the hypoxemia-induced decline in urine flow rate, GFR, RBF, FF as well as the increase in renal vascular resistance. The beneficial effects of theophylline could be mediated by its adenosine antagonistic properties.

In animal models as well as in humans, acute renal hypoxemia and ischemia can lead to renal insufficiency and failure. The model of post-occlusive renal ischemia has been used to elucidate the mechanisms mediating the hemodynamic changes associated with ischemia. In rabbits [1], rats [2] and dogs [3] post-occlusive renal ischemia is associated with renal vasoconstriction, a consequent decrease in renal blood flow (RBF) and an increase in renal adenosine content. The decrease in post-ischemic renal hemodynamic changes observed after the administration of theophylline, an adenosine antagonist [4], provided evidence for a causative role of adenosine in post-occlusive renal vasoconstriction [2, 5, 6] and suggested beneficial effects of theophylline (1–3 dimethylxanthine) in conditions associated with high renal adenosine content. Churchill and Bidani have hypothetized that extracellular renal adenosine mediates, at least in part, the decrease in glomerular filtration rate and filtration fraction in hypoxemic renal failure [7]. Recently Ramos-Salazar and Baines [8] demonstrated that 1,3-diprophyl-8-(2-amino-4-chlorophenyl) xanthine, a xanthine with adenosine antagonistic properties, reduces the effect of hypoxia in the isolated perfused rat kidney. No study has yet been performed, however, to determine whether theophylline could prevent the decrease in GFR associated with hypoxemia in clinical practice as well as in experimental conditions [9–11]. The protective effect of theophylline was investigated in anesthetized mechanically-ventilated newborn and adult rabbits subjected to normocapnic hypoxemia. We previously demonstrated that the response of this animal model to hypoxemia showed close similarities with the renal changes observed in hypoxemic neonates [9, 10]. Low-dose theophylline was used in order to achieve micromolar serum levels that inhibit adenosine interaction with its surface cellular membrane receptors without inhibiting cyclic AMP phosphodiesterase [4, 12]. The overall results clearly indicate that the hypoxemia-induced fall in glomerular filtration rate can be prevented by low-dose theophylline.

Methods

Experiments were performed in 22 newborn New Zealand White rabbits aged 3 to 12 days and weighing 73 to 156 g. Animals were born by spontaneous vaginal delivery and afterwards housed with the maternal rabbit and breast fed. Additional experiments were performed in 15 adult New Zealand White rabbits weighing 1.5 to 2.3 kg and maintained on a normal diet.

Experimental procedures in newborn rabbits

Newborn rabbits were initially anesthetized with 25 mg/kg 0.5% sodium pentobarbital injected intraperitoneally. Small doses of pentobarbital were subsequently administered as necessary. A tracheal cannulation allowed mechanical ventilation with a mixture of air and oxygen (Rodent ventilator, model 683, Harvard, Millis, Massachusetts, USA). The respiratory rate was kept constant at 40/min and tidal volume was adjusted for age and weight. A stretched polyethylene catheter (PE 10) was inserted into the right femoral artery for arterial blood sampling and monitoring of systemic blood pressure. A second catheter was similarly placed into the right femoral vein for solute infusion and drug administration. Surgical procedures and vascular cannulations were performed under stereoscopic magnifying-glass (Zeiss, Oberkochen, FRG). The bladder was catheterized for urine collection in preweighed microtest tubes.

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During the surgical procedure and subsequent experimental periods the body temperature of the newborn rabbits was kept constant at 38° to 38.5° C, using an infra-red lamp and a warming operating table. Throughout the experiment the heart rate (Sanborn 780-3 videoscope, Hewlett Packard, 78332, Palo Alto, California, USA), blood pressure (Statham P23 ID pressure transducer recorded on a Grass Polygraph, model 7B, Quincy, Massachusetts, USA) and oesophageal temperature (Digital thermometer, Poliak and Gramiger, EPFL, Lausanne, Switzerland) were continuously recorded.

After completion of the surgical procedure a priming dose of inulin (100 mg/kg) and para-aminohippuric acid (PAH, 1.25 mg/kg) was administered and a sustained infusion given to provide stable plasma levels of inulin (20 to 40 mg/100 ml) and PAH (0.2 to 1 mg/100 ml). The infusion rate was 1 ml/100 g/hr using a constant infusion pump (Perfusor EDL 2, Braun, Melsungen, FRG). The infusate contained per liter: 100 mmol NaCl, 5 mmol KCl, 50 mmol $NaHCO_3$, 50 g mannitol, 3 g inulin, 150 mg PAH. About one hour was spent for animal preparation and 90 to 120 minutes for subsequent equilibration. We previously demonstrated that blood pressure, heart rate, arterial pH, PO_2, PCO_2 and renal functions remain stable in this normoxemic animal model for up to three hours after completion of the equilibration period [13, 14].

The experimental protocol started with a one-hour control period (I). Hypoxemia was subsequently progressively induced within 20 to 30 minutes by slowly reducing the fractional inspiratory oxygen concentration. Our greatest concern was to preserve the stability of arterial blood pressure during hypoxemia. The hypoxemic period (II) lasted for one hour. Each animal acted as his own control. Two timed urinary collections of 30 minutes each were obtained during both the control and the hypoxemic period. Blood samples (0.4 ml) were withdrawn at the midpoint of alternate urinary collection periods. Eighty μl were used for immediate gas analysis, and hematocrit and protein levels determination. The remainder was centrifuged and the red blood cells were immediately reconstituted in up to 0.4 ml human albumin and returned to the animal. Plasma and urine samples were kept at 4°C for subsequent analysis. Three to five additional blood samples were obtained during the hypoxemic period to adjust and maintain the PaO_2 to 40 mm Hg.

Two different experimental protocol were applied to the newborn rabbits:

Group 1. Fourteen newborn rabbits aged 3 to 12 days (7.1 ± 0.7 days), weighing 73 to 155 g (114 ± 7 g) were used as a control group. These animals were without drug administration at the start of the hypoxemic period.

Group 2. Eight newborn rabbits aged 5 to 9 days (7.1 ± 0.4 days) weighing 88 to 156 g (123 ± 8 g), received a single i.v. dose of 0.6 mg/kg aminophylline (Euphyllin R, Byk Gulden, Konstanz, FRG), that is to say, 0.5 mg/kg theophylline (theophylline: 1 mg = 5.55 μmol. One mg of aminophylline ethylenediamine contains 0.81 mg of theophylline). The xanthine was diluted in 0.1 ml/100 g of a saline vehicle and slowly infused (5 min) at the end of period (I). During the hypoxemic period (II) 3 to 6 samples were obtained for serum theophylline determination in 6 animals. At the end of period II, the renal PAH extraction ratio (EPAH) was assessed in 5 out of 8 aminophylline-treated hypoxemic animals. Following a small laparotomy a fine needle was inserted into the left renal vein and venous blood was slowly extracted (approximately 0.04 ml/min) by a constant extraction pump (Perfusor EDL, Braun, Melsungen, FRG).

Previous data obtained in our laboratory indicated that the mean EPAH was 54.6 ± 3.7 and 45.7 ± 4.6% in 15 normoxemic and 8 hypoxemic newborn rabbits, respectively [10].

Experimental procedures in adult rabbits

Animal preparation. Animals were anesthetized with 30 mg/kg i.v. Na pentobarbital. Supplemental doses were given as required to maintain a surgical level of anesthesia. Tracheal cannulation allowed mechanical ventilation (Harvard apparatus respirator). Right femoral vessels were catheterized by polyethylene tubings for arterial blood sampling, monitoring of arterial blood pressure and intravenous solute infusion. The body temperature was maintained at 38°C using a warming operating table. The blood pressure was continuously recorded (Statham P23 ID pressure transducer recorded on a Gould electronics polygraph, Cleveland, Ohio, USA). After completion of the surgical procedure, priming doses of inulin (100 mg/kg) and PAH (0.6 mg/kg) were administered; their serum levels were maintained constant by infusing 24 ml/kg/hr of a solution containing per liter : 100 mmol NaCl, 6 mmol KCl, 50 mmol $NaHCO_3$, 50 g mannitol, 20 g inulin, 5 g PAH. Approximately 30 minutes were spent for animal preparation and 60 to 90 minutes for subsequent equilibration.

Effects of intravenous low-dose theophylline in hypoxemic adult rabbits (group 3). In 7 animals (1.65 to 2.25 kg) renal function was assessed during a 20 minute control period (I) followed by a 20 minute hypoxemic period (II).

Theophylline (2.4 mg i.v.) was subsequently slowly infused at the start of a third 20 minute hypoxemic period (III). During the experiment, timed urinary collections of 10 minutes each were obtained with blood samplings at the midpoint of each period. In 6 out of 7 animals renal venous blood was withdrawn at the end of period III for measuring the renal PAH extraction ratio. Previous studies conducted in our laboratory in 29 normoxemic and 21 hypoxemic adult rabbits demonstrated EPAH of 92.2 ± 1.9 and 90 ± 2.7%, respectively. Serum levels of theophylline were determined at 10 and 20 minutes after theophylline infusion in 6 out of 7 animals.

Effect of low-dose theophylline infused intra-renally in adult rabbits (group 4). In 8 adult rabbits (1.5 to 2.3 kg) the previous animal preparation was associated to two additive procedures: 1) separate catheterization of the ureters and 2) insertion of a fine needle in the left renal artery. The latter was infused with 0.6 ml/hr of a saline vehicle during a 30 minute normoxemic control period and subsequently with 33 μg/min aminophylline (= 27 μg/min theophylline) diluted in the same saline vehicle during a 30 minute hypoxemic period. The greatest concern was to avoid injury of the sympathetic renal nerves. Renal functions (urine flow rate, glomerular filtration rate, renal blood flow, renal vascular resistance and filtration fraction) of the two kidneys were studied separately during normoxemia and hypoxemia. In 5 animals serum theophylline levels were determined at the tenth, twentieth, and thirtieth minute of the hypoxemic period. At the end of the experiments the rabbits were killed with a lethal dose of Na pentobarbital. The clearances (C) of inulin and PAH and the extraction of PAH were calculated from standard formulae. Renal blood flow (RBF),

Table 1. Physiological conditions during a one-hour control period (I) and a subsequent one hour hypoxemic-period (II) in untreated (group 1) and theophylline-treated (group 2) newborn rabbits

Groups (*N*)	1 (14)		2 (8)	
Periods	I	II	I	II
PaO_2 *mm Hg*	129 ± 7	39.9 ± 1.6[a]	118 ± 4	41.9 ± 1.6[a]
$PaCO_2$ *mm Hg*	38.7 ± 0.9	37.3 ± 0.9	40.5 ± 1.3	40.9 ± 1.9
pH	7.469 ± 0.008	7.468 ± 0.013	7.437 ± 0.013	7.417 ± 0.015
Hct. *%*	30.6 ± 1.2	29.2 ± 1.2[a]	30.3 ± 1.4	27.5 ± 0.8[a]
Prot *g/liter*	29.6 ± 1.1	29.9 ± 1	32.3 ± 1.5	31.0 ± 1.6
MBP *mm Hg*	33.3 ± 1.5	33.0 ± 1.4	33.8 ± 1.8	33.6 ± 2

Group 2 received 0.5 mg/kg i.v. theophylline at the onset of the hypoxemic period. Values are means ± SEM. Abbreviations are: MBP, mean blood pressure; Prot, protid level.

[a] Levels of probability when comparing periods I to II: $P < 0.01$

Table 2. Hypoxemia-induced renal changes in newborn rabbits either untreated (group 1) or administered 0.5 mg/kg i.v. theophylline (group 2) at the onset of the hypoxemic period (II)

Groups (*N*)	1 (14)		2 (8)	
Periods	I	II	I	II
V *ml/kg/min*	0.081 ± 0.007	0.083 ± 0.008	0.073 ± 0.003	0.100 ± 0.004[b]
GFR *ml/kg/min*	2.04 ± 0.15	1.60 ± 0.18[b]	2.58 ± 0.31	2.75 ± 0.28
RBF *ml/kg/min*	21.4 ± 1.7	19.5 ± 1.7	24.4 ± 2.3	23.2 ± 2
RVR *mm Hg/ml/kg/min*	1.65 ± 0.12	1.84 ± 0.14[a]	1.47 ± 0.15	1.51 ± 0.15
FF *%*	14.2 ± 0.9	11.7 ± 0.8[b]	15.8 ± 1.8	16.8 ± 1.5

Values are means ± SEM.

Levels of probability when comparing periods I to II: [a] $P < 0.05$; [b] $P < 0.01$.

renal vascular resistance (RVR) and filtration fraction (FF) were derived from the following equations:

RBF (ml/kg/min) = C PAH /(E PAH × (1 − Hct)),
where Hct = hematocrit
RVR (mm Hg/ml/kg/min) = blood pressure/RBF
FF (%) = (GFR/RPF) × 100,
where GFR = glomerular filtration rate = C inulin,
RPF = renal plasma flow = C PAH/EPAH

Analytical methods

The urine volume was calculated from the change in weight of preweighed tubes without correction for specific gravity (Analytical balance, Mettler, Greifensel, Zurich, Switzerland). Arterial blood for pH, PCO_2, PO_2 and hematocrit determination was collected anaerobically in heparinized capillary tubes. Blood gas determination was performed using a pH/blood gas analyzer (Gas analyser 168, Corning Halsted, Essex, UK). Automatic anthrone [15] and Bratton and Marshall [16] methods were used for the determination of inulin and PAH concentrations (Autoanalyzer II, Technicon Instrument Corporation, Tarrytown, New York, USA). Serum theophylline concentrations were measured by high performance liquid chromatography according to a previously described method [17]. Statistical analysis [18] was performed[2] by paired or unpaired *t*-test, one-way analysis of variance or two-ways analysis of variance for repeated measurements (group 3). All values are expressed as means ± SEM.

[2] All computations were done using the Triomphe software which has been developed by Mr. P. d'Athis at the Department of Medical Informatics in Dijon Hospital, 21034 Dijon Cedex, France.

Results

In newborn rabbits the mean PAH extraction ratio (EPAH) was not significantly different between normoxemic (54.6 ± 3.7%), untreated hypoxemic (45.7 ± 4.6%) [10], and theophylline-treated hypoxemic animals (50.0 ± 6.8%). EPAH was similar in normoxemic (92.2 ± 1.9%), hypoxemic (90 ± 2.7%) and theophylline-treated hypoxemic adult rabbits (91.3 ± 2%). RBF was subsequently calculated from the respective mean EPAH values in each group.

Newborn rabbits

Postnatal age, weight and PaO_2 during the control and the hypoxemic periods were not statistically different between the two groups of newborn rabbits.

Effects of hypoxemia (group 1). Arterial PCO_2, pH, protein levels and blood pressure remained constant during hypoxemia (Table 1). As a consequence of repeated blood sampling the hematocrit decreased slightly during the hypoxemic period. Hypoxemia was associated with a significant decline in GFR (−22 ± 6%), FF(−17 ± 3%), whereas RVR increased significantly (+13 + 6%) (Table 2, Fig. 1). The slight increase in urine flow rate (+4 ± 8%) and the decrease in RBF (−9 ± 5%) did not reach statistical significance.

Effect of low-dose theophylline in hypoxemic animals (group 2). Arterial PCO_2, pH, protein levels and mean blood pressure did not vary from period I to period II (Table 1). Hematocrit

Table 3. Renal functions and hemodynamics values in 7 adult rabbits (group 3) during a control period I (PaO_2 = 125 ± 23 mm Hg) and two subsequent hypoxemic periods II (PaO_2 = 41.2 ± 1.8 mm Hg) and III (PaO_2 = 40.4 ± 1.5 mm Hg)

Periods	I	II	III
V *ml/kg/min*	0.569 ± 0.086	0.421 ± 0.062	0.586 ± 0.117
GFR *ml/kg/min*	3.95 ± 0.46	2.75 ± 0.24[c]	3.23 ± 0.32[a]
RBF *ml/kg/min*	35.2 ± 5	24.8 ± 2.9[c]	25.9 ± 3.1
RVR *mm Hg/ml/kg/min*	2.44 ± 0.42	3.25 ± 0.41	3.13 ± 0.46
FF %	20.3 ± 2.5	19.6 ± 1.8	22.5 ± 2.3[b]

2.4 mg theophylline were infused at the start of period III.
Values are means ± SEM.
Levels of probability when comparing periods I to II and period II to III: [a] $P < 0.05$; [b] $P < 0.025$; [c] $P < 0.01$.

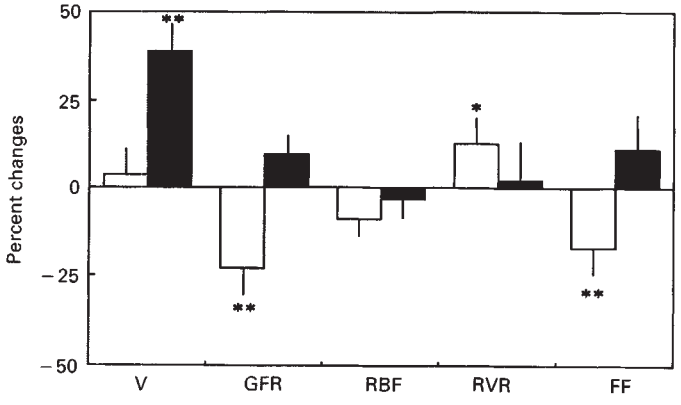

Fig. 1. *Percent hypoxemia-induced changes in V, GFR, RBF, RVR and FF in untreated (□) and theophylline-treated (■) hypoxemic newborn rabbits.* Levels of probability when comparing the percent changes in the two groups: *$P < 0.05$; ** $P < 0.01$.

slightly decreased (−8.5 ± 2.3%). Urine flow rate increased significantly during the hypoxemic period (+38 ± 7%) while GFR, RBF, RVR and FF did not change significantly in response to the hypoxemic stress (Table 2, Fig. 1). The mean serum theophylline levels in the first and the second halves of the hypoxemic period were similar (0.655 ± 0.062 and 0.617 ± 0.066 μg/ml, respectively).

Adult rabbits

Effect of low-dose theophylline in previously hypoxemic adult rabbits (group 3). Hypoxemia (PaO_2 = 41.2 ± 1.8 mm Hg) in period II was associated with significant decreases ($P < 0.01$) in GFR (−27 ± 6%) and RBF (−29 ± 6%) (Table 3). Though RVR increased by 44 ± 19% and urine flow rate decreased by 21 ± 12%, these changes did not reach statistical significance. Filtration fraction remained stable. Systemic infusion of theophylline at the onset of period III (PaO_2 = 40.4 ± 1.5 mm Hg) was followed by a significant increase in GFR (+17 ± 5%) and FF (+14 ± 3%). Non-significant increases in urine flow rate (+38 ± 18%) and RBF (+4 ± 4%) were observed. In spite of a significant improvement, GFR remained significantly lower in period III (−14 ± 9%) as compared to the normoxemic control period (Table 3).

Throughout the three successive periods there was no significant change in arterial pH (7.37 ± 0.04; 7.35 ± 0.03; 7.35 ± 0.03), $PaCO_2$ (42.8 ± 4; 44.3 ± 4; 45 ± 3 mm Hg), hematocrit (35.3 ± 1.3; 34.7 ± 1.9; 35.3 ± 1.9%) and mean blood pressure (86 ± 4; 81 ± 4; 81 ± 4 mm Hg). Mean serum theophylline levels of 1.365 ± 0.028 and 1.132 ± 0.08 μg/ml ($P < 0.05$) were observed 10 and 20 minutes after starting the infusion.

Effect of low-dose intra-renal theophylline (group 4). In these animals the decrease in PaO_2 from 114 ± 6 mm Hg to 37 ± 2 mm Hg was associated with : a slight increase in $PaCO_2$ (from 32 ± 3 mm Hg to 35 ± 3 mm Hg; $P < 0.01$); a slight decrease in arterial pH (from 7.453 ± 0.021 to 7.401 ± 0.014; $P < 0.01$), blood pressure (from 83 ± 2 to 80 ± 2 mm Hg; $P < 0.05$) and hematocrit (from 35.6 ± 1.2 to 32.6 ± 2.8%; $P < 0.01$). Urine flow rate, GFR, RBF, RVR and FF of the two kidneys were similar during the normoxemic period (Table 4). Following hypoxemia, GFR and RBF decreased significantly in the two kidneys with a significant increase in RVR. Yet, the theophylline infused kidney showed significantly higher values of urine flow rate, GFR, RBF, FF and lower values of RVR than the control kidney during the hypoxemic period (Table 4; Fig. 2). The mean serum theophylline levels during the hypoxemic period were 0.31 ± 0.09, 0.34 ± 0.06, 0.59 ± 0.07 μg/ml at the tenth, twentieth, and thirtieth minutes of hypoxemia, respectively. The mean concentration was significantly higher at the thirtieth minute than in the two previous samples ($P < 0.025$).

Discussion

The present study confirms our previous observation that normocapnic hypoxemia is associated with a significant fall in GFR in immature newborn rabbits with otherwise stable physiological conditions (blood pH, $PaCO_2$, mean blood pressure) [10]. This hypoxemia-induced drop in GFR is associated with concomitant decreases in filtration fraction and RBF and an increase in RVR. The newborn rabbit thus appears a valuable model for studying the hypoxemic renal insufficiency reported in immature animals [19, 20] as well as in human neonates [9]. Furthermore, as observed in lambs [20] the renal immaturity does not appear to aggravate the hypoxemia-induced decrease in GFR in newborn rabbits as compared to adult animals. The mechanisms underlying the hypoxemia-induced hemodynamic renal changes can be inferred from the present data. The stable plasma protein concentrations in our animals indicate that the changes in GFR were not related to variations in the plasma oncotic pressure. Changes in the ultrafiltration coefficient (K_f) during the hypoxemic period of either treated or untreated

Table 4. Renal functions of the two separated kidneys (group 4) during the normoxemic period (I) (PaO_2 = 114 ± 6 mm Hg) and the subsequent hypoxemic period (II) (PaO_2 = 37 ± 2 mm Hg)

Periods	Normoxemia		Hypoxemia	
Kidney	C	T	C	T
V *ml/kg/min*	0.330 ± 0.046	0.309 ± 0.054	0.140 ± 0.030[b]	0.230 ± 0.013[d]
GFR *ml/kg/min*	2.31 ± 0.27	2.23 ± 0.24	1.11 ± 0.22[b]	1.66 ± 0.15[a,d]
RBF *ml/kg/min*	19.4 ± 1.4	18.8 ± 1.3	11.5 ± 1.3[b]	13.5 ± 1.2[b,d]
RVR *mm Hg/ml/kg/min*	4.23 ± 0.33	4.35 ± 0.32	6.93 ± 0.81[b]	5.88 ± 0.62[a,d]
FF %	18.5 ± 1.6	18.7 ± 1.8	16.1 ± 0.8	18.4 ± 1.1[c]

Abbreviations are: C, right control kidney; T, left theophylline (27 μg/min) infused kidney.
Levels of probability when comparing period I to II: [a] $P < 0.01$; [b] $P < 0.005$.
Levels of probability when comparing the control and the theophylline infused kidney during the same period: [c] $P < 0.025$; [d] $P < 0.005$.

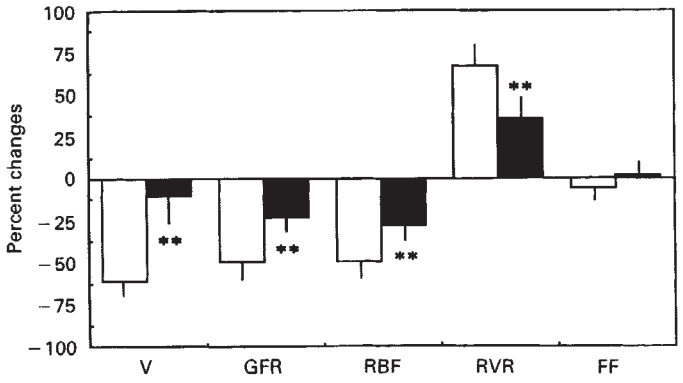

Fig. 2. *Percent hypoxemia-induced changes in renal functions (V, GFR, RBF, RVR, FF) of the right control kidney (□) and the left theophylline (27 μg/min) infused kidney (■).* Levels of probability when comparing the percent changes of the two kidneys: $^{**}P < 0.01$.

animals also appear unlikely. This assumption is supported by: a) recent experimental data failing to demonstrate a fall in K_f at the early phase of renal ischemia [21]; b) the observation that a theophylline-induced increase in the diameter of isolated glomeruli [22] was only observed at theophylline concentrations (> 200 μg/ml) greatly exceeding the values observed in our animals. In the absence of changes in K_f, the hypoxemia-induced decrease in filtration fraction observed in newborn rabbits (group 1) could indicate relative postglomerular vasodilatation, while the hypoxemia-induced increase in RVR and decrease in GFR in newborn and adult rabbits suggest concomitant preglomerular vasoconstriction.

Low-dose intravenous theophylline (0.5 mg/kg), providing serum theophylline levels close to 0.7 μg/ml, completely prevented the hypoxemia-induced changes in GFR and renal hemodynamics in newborn rabbits. In mature animals intrarenal theophylline (27 μg/min) only partially prevented the hypoxemic renal changes (group 4). The arterial theophylline concentrations in the right and left kidneys can be derived from the systemic concentrations (femoral sampling), the theophylline infusion rate (27 μg/min) in the left renal artery and the left effective renal plasma flow (16.3 ± 1.3 ml/min). The following theophylline concentrations in the right and left renal arteries can be estimated: 0.31 and 2.05 μg/ml at the tenth minute; 0.34 and 2.08 μg/ml at the twentieth minute; 0.59 and 2.33 μg/ml at the thirtieth minute. The immature kidney was thus protected from the deleterious effect of hypoxemia by theophylline concentrations lower than those providing partial protection in the adult rabbit. The responses of the two hypoxemic animal models to theophylline are difficult to compare however, because the renal hemodynamic changes induced by hypoxemia were not identical. The precise mechanism by which low-dose theophylline blunts the renal hemodynamic effects of hypoxemia is speculative. It cannot be ascribed to an antagonism with renal cyclic AMP phosphodiesterase because the latter needs millimolar theophylline concentrations [13, 23], whereas only micromolar serum concentrations were achieved in our experiments.

Our results suggest that theophylline could act by antagonizing renal endogenous adenosine, a putative mediator of the hypoxemia-induced renal changes. Recent observations support this hypothesis: 1) adenosine is involved in the renal hemodynamic changes induced by renal ischemia in the whole animal model [2, 5] and by renal hypoxemia in the isolated rat kidney [8]; 2) low-dose theophylline prevents the renal effects of exogenous adenosine and of renal ischemia [2, 5, 6, 24]; 3) micromolar theophylline concentrations, as obtained in our experiments, are sufficient to antagonize the renal effects of adenosine [2, 4, 25]; 4) unpublished observations obtained in our laboratory indicate that 0.5 mg/kg enprofylline (3-propylxanthine), a xanthine derivative devoid of adenosine antagonistic properties [4], does not prevent the hypoxemia-induced renal changes in newborn rabbits. The nature of the renal hemodynamic changes induced by hypoxemia supports the hypothesis that they could be mediated by adenosine. As mentioned above, the decrease in GFR and the concomitant decrease in FF induced by hypoxemia in newborn rabbits could be the result of preglomerular vasoconstriction and postglomerular vasodilatation. While the latter effect could be due to the postglomerular vasodilating effect of adenosine [8, 26, 27], a preglomerular vasoconstriction could be mediated by the combined effect of angiotensin II and adenosine. Renal adenosine has indeed been shown to increase the preglomerular vascular response to angiotensin II in conditions such as ischemia associating high renal contents of adenosine and angiotensin II [28, 29]. That the renin-angiotensin system was activated in our hypoxemic conditions was previously demonstrated both in our

study in adult [11] and immature animals [30]. Because theophylline has been shown to stimulate, rather than depress, the renin-angiotensin system in various experimental conditions [24], its beneficial effect on the hypoxemic kidney is more likely to be due to its antagonistic properties with adenosine. Whatever the mechanisms of its action, the beneficial effect of low-dose theophylline in preventing the renal vasoconstriction induced by hypoxemia and ischemia in developing and mature animals could have far-reaching clinical consequences. Recent observations on the effects of adenosine antagonists in various experimental conditions of acute renal failure (myoglobunuria, endotoxin infusion, hyperosmolar contrast agents or glycerol administration) confirm both the potential usefulness of low-dose theophylline to prevent an incipient renal insufficiency and the possible role of adenosine as a mediator of the decrease in GFR [31–34].

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